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ORIGINAL RESEARCH PAPER

Determination of aflatoxin B_1 levels in Iranian and Indian spices by ELISA method

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Abstract

This study was carried out to detect the presence of aflatoxin B_1 (AFB_1) in 36 samples of spices from Iran and India that include chilli powder ($n = 12$), black pepper powder ($n = 12$) and whole black pepper ($n = 12$). Enzyme-linked immunosorbent assay was applied to analyse AFB_1 in the samples. All the analyses were done twice. AFB_1 was found in all the spices samples, the concentration of AFB_1 in Iranian samples was ranged from 63.16 to 626.81 ng/kg and in Indian samples was ranged from 31.15 to 245.94 ng/kg. The mean of AFB_1 concentration in the chilli powder was significantly higher ($p < 0.05$) than the whole and powdered black pepper. However, none of the samples exceeded the maximum prescribed limit, that is 5000 ng/kg (5 μg/kg) of European Union regulations for AFB_1. Although, the present research was not a comprehensive study; however, it provides valuable information on AFB_1 levels in Iranian and Indian spices.

Keywords

Aflatoxin B_1, black pepper, chilli, ELISA, India, Iran

History

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Introduction

Aflatoxins are recognized to be ubiquitous contaminants of food throughout the developing world (Kensler et al., 2011). The main aflatoxins are identified to be B_1, B_2, G_1 and G_2 which are highly toxic, mutagenic, estrogenic, immunosuppressive, tremorogenic, teratogenic and carcinogenic (Ardic et al., 2008; O'Riordan & Wilkinson, 2008). Aflatoxins are produced by different *Aspergillus* species, like *A. flavus*, *A. parasiticus*, *A. pseudotamarii*, *A. bombycis*, *A. ochraceoroseus*, *Emericella venezuelensis*, and *A. nomius* (Kamkar et al., 2014a, Kamkar et al., 2014b). Among them, aflatoxin B_1 (AFB_1) is the most potent case of human hepatocellular carcinoma; hence, the International Agency for Research on Cancer (IARC) of World Health Organization (WHO) classified AFB_1 into the primary group of carcinogenic compounds (Tavakoli et al., 2013). Aflatoxins can contaminate plant products e.g. cereals, oilseeds, coffee, grapevine, dried fruits and various spices (Ardic et al., 2008; Colak et al., 2006). Spices are esoteric food adjuncts that are used for flavour, colour, aroma and preservation of food or beverages for thousands of years. The most important spices are red pepper, black pepper, turmeric cinnamon, ginger and cumin. Chilli also named red pepper belongs to the genus Capsicum (dried from of *Capsicum annum* L.) is the second largest consumed spice throughout the world, after black pepper (Golge et al., 2013). Paprika is a spice normally coming from red pepper (*Capsicum annum* L.), obtained by grinding pods from dried fruits (Shundo et al., 2009). White and black pepper (*Piper nigrum* L.) are produced by drying the unripe green pepper berries while white pepper is produced by soaking the matured red peppers berries in water for about a week (Chai & Elie, 2013). Some countries have carried out studies about acceptable levels of aflatoxins in spices and other agriculture products. European Union (EU) has established maximum tolerable limits for AFs in spices as 10 μg/kg for total aflatoxins ($B_1 + B_2 + G_1 + G_2$) and 5 μg/kg for AFB_1 (Iqbal et al., 2010a). However, spices may become contaminated with aflatoxins during pre-harvest, post-harvest, storage or transport as they are sensitive to aflatoxins contamination depending on temperature, humidity, drying and processing conditions. Jalili et al. (2010) reported the effect of gamma ray treatments on reducing mycotoxin concentration in different foods. Several methods have been described for determination of aflatoxin, including the thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA) (Kamkar et al., 2014b). ELISA is well suited for the rapid, routine diagnostic application of aflatoxin detection (Mozaffari Nejad et al., 2013).

The aim of this study was to investigate and compare the presence of AFB_1 in different samples of spices including black pepper and red pepper in Iran and India.

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Table 1. The occurrence of AFB_1 in spices samples in Iran and India.

Country	Sample category	Sample (*n*)	Minimum (ng/kg)	Maximum (ng/kg)	Mean ± SE[a] (ng/kg)
Iran	Chilli powder	6	255.52	626.81	$414.27^{a} \pm 49.07$
	Black pepper powder	6	100.75	535.84	$243.34^{b} \pm 67.76$
	Whole black pepper	6	63.16	266.79	$187.57^{b} \pm 32.79$
	Total	18	63.16	626.81	281.73 ± 36.59
India	Chilli powder	6	64.63	245.94	$153.11^{a} \pm 27.32$
	Black pepper powder	6	48.35	174.68	$123.70^{b} \pm 18.20$
	Whole black pepper	6	31.15	44.12	$36.52^{b} \pm 2.31$
	Total	18	31.15	245.94	104.44 ± 15.94

[a,b]Mean ± SE (standard error) with different letters is significantly different.

Materials and methods

Samples

In June 2012, a total of 36 samples of spices from two countries of Iran and India which, in total of India was 18 that were commercially available and randomly purchased from three popular markets in Hyderabad, Andhra Pradesh Province in India and 18 samples of spices commercialized were randomly purchased from three popular herbal shops in Qaemshahr, Mazandaran Province in Iran. The spice samples included whole black pepper ($n = 12$), black pepper powder ($n = 12$) and chilli powder ($n = 12$). All samples were powdered with Moulinex blender (Moulinex Products Co., Caen, France). A 100 g of each sample was stored at 4–6 °C in sealed plastic bags until the analysis (Ardic et al., 2008; Colak et al., 2006). All samples were analysed in duplicate.

Analysis of AFB_1 by ELISA

The quantitative analysis of AFB_1 in the samples was performed based on a competitive enzyme immunoassay by using RIDASCREEN® AFB_1 30/15 (Art. No: R1211; R-Biopharm, Darmstadt, Germany) test kit. Preparation of the samples and ELISA test were performed according to the method described by R-Biopharm GmbH (2010).

Samples preparation

Samples preparation and separation with aflatoxin column were preformed according to the instructions of the test kit manual (Ridascreen AFB_1 30/15) (R-Biopharm GmbH, 2010). Twenty-five millilitre of methanol (70%) was added to 5 g of spices. Afterwards, the samples were vigorously shaked for 3 min manually. The achieved extract was filtered through a filter paper and diluted with distilled water (1:1). At last, 50 µl of the diluted filtrate per well was used in the test.

ELISA test procedure

According to Ridascreen AFB_1 30/15 (Art No.: 1211) test kit manual, 50 µl of the standard solution or prepared sample in duplicate was added to the wells of microtitre plate. Then, 50 µl of the enzyme conjugate and 50 µl of the anti-aflatoxin antibody solution were added to each well, mixed gently and incubated for 30 min at room temperature (20–25 °C). Liquid was removed from wells by tapping the wells upside down vigorously against the absorbent paper; the wells were then washed by a washing buffer (250 µl) twice. After the washing step, 100 µl of substrate/chromogen solution was added to each well, mixed gently and incubated for 15 min at room temperature (20–25 °C) in a dark place. Finally, 100 µl of the stop solution (1 N H_2SO_4) was added to each well and the absorbance was measured at 450 nm in ELISA plate reader.

Statistical analysis

The data were analysed by IBM SPSS Statistics software (version 16; IBM SPSS Inc., Chicago, IL). Moreover, to evaluate the differences in mean values, three groups of samples were used for *t*-test and analysis of variance was used for independent samples. The differences among the mean values were found to be significant at $p \leq 0.05$.

Results and discussion

The occurrence and levels of AFB_1 in spice samples consisting of chilli powder, black pepper powder and whole black pepper, which were collected from Iran and India, are presented in Table 1. AFB_1 was found in all spices samples of Iran and India. The ranges of AFB_1 contamination were 31.15–245.94 ng/kg and 36.16–626.81 ng/kg for India spice samples and Iran spice samples, respectively. Also, none of the samples exceeded the EU limit of 5 µg/kg for AFB_1. The mean of AFB_1 concentration in the chilli powder from Iran and India was significantly higher ($p < 0.05$) than the findings in samples whole black pepper and black pepper powder. However, no significant difference was observed in the mean AFB_1 concentration in the whole black pepper and black pepper powder.

Spices are produced and consumed in developing and developed countries (Paterson, 2007). Among aflatoxins, AFB_1 is the most frequent toxin in spices with high levels and red pepper samples are the most frequently contaminated substrate (Ardic et al., 2008). A number of surveys of AFB_1 contamination in spices are reported in the other studies. As shown in Table 2, several studies (Abdulkadar et al., 2004; Bircan, 2005; Colak et al., 2006; Fazekas et al., 2005; Golge et al., 2013; Iqbal et al., 2011; Jalili & Jinap, 2012; Kursun & Mutlu, 2010; Martins et al., 2001; Musaiger et al., 2008; Omurtag et al., 2002; O'Riordan & Wilkinson, 2008; Ozbey & Kabak, 2012; Reddy et al., 2001, 2011; Romagnoli et al., 2007; Santos et al., 2010; Shundo et al., 2009; Tosun & Arslan, 2013) reported the AFB_1 contamination in different kinds of spices.

Table 2. Incidence and levels of AFs in different kinds of spices reported in previous studies.

Country	Products	No. of samples	Positive *n* (%)	Method	Mycotoxin	Range (μg/kg)	Reference
India	Chilli	182	107 (59)	ELISA	AFB_1	<10–969	Reddy et al., 2001
Portugal	White pepper	7	3 (43)	HPLC	AFB_1	1.25–5.0	Martins et al., 2001
	Chilli	8	3 (38)			1.5–2.2	
Turkey	Red pepper	26	17 (65)	HPLC-FD	AFB_1	0.6–56	Omurtag et al., 2002
Qatar	Mixed spices powder	6	5 (84)	HPLC	AFs	0.16–5.12	Abdulkadar et al., 2004
Turkey	Paprika	30	27 (90)	HPLC-FD	AFs	0.5–124.6	Bircan, 2005
					AFB_1	0.5–116.4	
	Chilli powder	15	15 (100)		AFs	1.8–85.9	
					AFB_1	1.6–80.4	
	Ground black pepper	15	4 (27)		AFs	0.3–2.3	
					AFB_1	0.3–2.3	
Hungary	Black pepper	5	1 (17)	HPLC-FD	AFB_1	0.46	Fazekas et al., 2005
	White pepper	5	0			ND	
Turkey	Red-scaled pepper	30	13 (44)	ELISA	AFB_1	1.9–35.5	Colak et al., 2006
	Black pepper	24	2 (9)			9.8–10.3	
Italy	Black pepper	11	0	ELISA	AFB_1	ND	Romagnoli et al., 2007
	Chilli	11	5 (46)			5–27	
Bahrain	Black pepper powder	4	4 (100)	HPLC	AFs	10.3–13.8	Musaiger et al., 2008
					AFB_1	0.8–1.3	
	Red chilli powder	6	6 (100)	HPLC	AFs	25.5–30	
					AFB_1	23.4–27.6	
Ireland	Turmeric	10	4 (40)	HPLC-FD	AFs	0.81–16.40	O'Riordan & Wilkinson, 2008
	Pepper	30	4 (14)			0.42–3.24	
	Curry powder	20	3 (15)			0.5–9.1	
Brazil	Paprika	70	15 (22)	HPLC-FD	AFs	1.7–7	Shundo et al., 2009
			43 (62)		AFB_1	0.5–7.3	
Spain	Paprika	64	38 (59)	HPLC-FD	AFs	0.5–7.25	Santos et al., 2010
	Chilli powder	35	14 (35)			0.5–2.49	
Turkey	Red pepper	34	34 (100)	ELISA	AFs	3.55–9.55	Kursun & Mutlu, 2010
Pakistan	Whole chilli	78	26 (33)	HPLC-FD	AFs	0.00–81.5	Iqbal et al., 2011
	Ground chilli	78	31 (40)			0.00–84.8	
Malaysia	Chilli	8	8 (100)	ELISA	AB_1	0.58–3.5	Reddy et al., 2011
Malaysia	Dried chilli	80	52 (65)	HPLC-FD	AFB_1	0.2–56.61	Jalili & Jinap, 2012
Turkey	Black pepper powder	23	7 (31)	HPLC-FD	AFs	0.13–0.46	Ozbey & Kabak, 2012
					AFB_1	0.13–0.42	
Turkey	Black pepper	6	4 (67)	ELISA	AFB_1	24.6–30	Tosun & Arslan, 2013
	Red pepper	8	6 (75)			23.4–46.6	
Turkey	Chilli	182	150 (83)	HPLC-FD	AFB_1	0.24–165	Golge et al., 2013

HPLC-FD: HPLC fluorescence detection.

This study was the second report on AFB_1 in spice samples in Iran while several reports have been presented by researchers on dairy products, rice and pistachio in this country. In the first report by Salari et al. (2012) in Sabzevar (a city in Razavi Khorasan province, Iran), they were reported among a total of 36 samples of red pepper, the incidence of AFB_1 and ochratoxin A that was 25 (69.4%) and 6 (16.7%) within the range of 0.4–14.5 and 0.74–2.17 μg/kg, respectively, as it was determined by HPLC. There are several studies have reported high occurrence of aflatoxin but low mean concentration. According to Ozbey and Kabak (2012) from Turkey, who have analysed 22 red chilli powder samples and found that 63.6% of samples contained AFs at detectable levels and three (13.6%) red chilli powder were found above the EU regulatory limit for AFB_1. From Brazil, Shundo et al. (2009) have reported that 82.9% samples of paprika were positive with AFs, and AFB_1 was detected in 61.4% at levels ranging from 0.5 to 7.3 μg/kg with mean concentration of 3.4 μg/kg. In a previous survey by Paterson (2007) from Pakistan, reported that 13 (100%) samples of 13 chilli samples had higher concentration of total aflatoxin and eight samples were higher than the EU maximum limit of 10 μg/kg and also 100% samples were found contaminated with AFB_1 with range level of 0.1–96.2, which is similar with our results. From Pakistan, Hussain et al. (2012) have observed that 12 (66.7%) out of total 18 samples of chillies contaminated with AFB_1. In a previous study by Iqbal et al. (2010b) from Pakistan reported that the occurrence and concentration range of AFB_1 in the ground ($n = 22$) and whole ($n = 22$) chilli samples were investigated by HPLC. They reported that ground chilli samples were the highest mean concentration of AFB_1 (32.20 ± 9.15 μg/kg) compared with whole chillies (24.69 ± 8.19 μg/kg). Also, the number of whole chillies with at least 5 μg/kg AFB_1 (the EU limit) was 16 (73%) with a range of 0.00–96.3 μg/kg; 19 (86.4%) of the ground chilli samples (range of 0.00–89.56 μg/kg) were contaminated with AFB_1. The mean contamination of AFB_1 was highest in ground chillies. Several studies have been performed in different countries for investigating the aflatoxin contamination in spices. Hayaloglu et al. (2005) from Turkey reported that in just 1 of 40 (2.5%) red-blackish ground peppers (isot) samples contained AFB_1 level 3 μg/kg, but our results were higher than this result. Also in the same study from Pakistan which was performed by Ahmad and Ahmed (1995) reported that 117 (66%) of 176 red pepper samples were contaminated with AFB_1 and the average AFB_1 level was 25 μg/kg in seven

red pepper samples. In another study which were performed by Fufa and Urga (1996) in Ethiopia, reported by TLC method that in screening AFs in ground red pepper, they found 8 of 60 samples (13.3%) collected from markets, shops and storage facilities were contaminated with AFB_1 in concentrations of 250–525 μg/kg. Gurbuz et al. (1999) examined 75 red ground pepper samples and detected AFB_1 at levels between 0.25 and 10 μg/kg in 32% of the samples. Furthermore, in Morocco, 14 red paprika pepper samples were screened for aflatoxin contamination and 14 (100%) samples contained AFB_1. The aflatoxin of spice was in the range of 2.88–5.40 μg/kg (Zinedine et al., 2006).

Conclusion

This study evaluated AFB_1 contamination in spices such as chili powder, black pepper powder and whole black pepper obtained from markets in Iran and India. Although none of the samples exceeded the EU limit (5 μg/kg), all of them were contaminated with AFB_1. Contamination of the samples in such a high level could be a potential hazard for health of consumers. It is important to inspect and control spices for presence of mycotoxins in a regular manner. The spices with high levels of mycotoxins should not be allowed for human consumption by the public health authorities.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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